

## New England Biolabs Certificate of Analysis

**Product Name:** Pacl  
**Catalog Number:** R0547S  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pNEB193 DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10167784  
**Expiration Date:** 10/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0547S/L/V v2.0

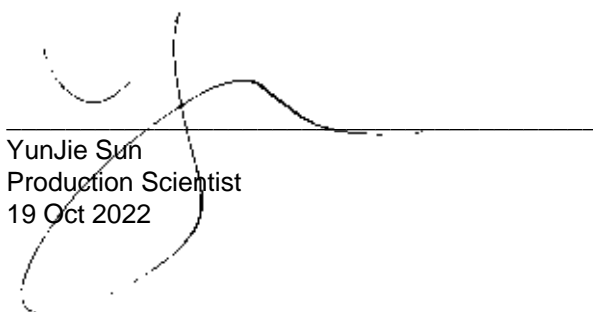
| Pacl Component List |                              |            |                      |
|---------------------|------------------------------|------------|----------------------|
| NEB Part Number     | Component Description        | Lot Number | Individual QC Result |
| R0547SVIAL          | Pacl                         | 10167783   | Pass                 |
| B7024AVIAL          | Gel Loading Dye, Purple (6X) | 10163561   | Pass                 |
| B6004SVIAL          | rCutSmart™ Buffer            | 10165691   | Pass                 |

| Assay Name/Specification  | Lot # 10167784 |
|---|----------------|
| <b>Exonuclease Activity (Radioactivity Release)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of Pacl incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.     | Pass           |
| <b>Functional Testing (15 minute Digest)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pNEB193 DNA and 1 µl of Pacl incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.  | Pass           |
| <b>Non-Specific DNase Activity (16 Hour)</b><br>A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pNEB193 DNA and a minimum of 100 units of Pacl incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass           |
| <b>qPCR DNA Contamination (E. coli Genomic)</b><br>A minimum of 10 units of Pacl is screened for the presence of E. coli genomic DNA  | Pass           |

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|---|----------------|
| <p>using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is <math>\leq 1</math> E. coli genome.</p>   |                |
| <p><b>Endonuclease Activity (Nicking)</b><br/>A 50 <math>\mu</math>l reaction in rCutSmart™ Buffer containing 1 <math>\mu</math>g of supercoiled PhiX174 DNA and a minimum of 30 units of PacI incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p> | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>PacI is &gt;95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <p><b>Blue-White Screening (Terminal Integrity)</b><br/>A sample of pNEB193 vector linearized with a 10-fold excess of PacI, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</p>  | <b>Pass</b>    |
| <p><b>Ligation and Recutting (Terminal Integrity)</b><br/>After a 10-fold over-digestion of pNEB193 DNA with PacI, ~75% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with PacI.</p>   | <b>Pass</b>    |

This product has been tested and shown to be in compliance with all specifications.

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19 Oct 2022



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